LA-UR-77-1244

CONF-770324--2

TITLE:

FLOW-SYSTEM MULTIANGLE LIGHT-SCATTERING STUDIES:

A PROGRESS REPORT

AUTHOR(S):

G. C. Salzman, D. E. Burger, M. Bartholdi, and B. J. Price

SUBMITTED TO:

In: Proceedings of the Third International Symposium on Pulse-Cytophotometry, held in Vienna, Austria (March 30-April 1, 1977); European Press, Ghent, Belgium

los alamos
scientific laboratory
of the University of California
LOS ALAMOS, NEW MEXICO 87545

An Affirmative Action/Equal Opportunity Employer

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

Form No. 836
St. No. 2629
1/75

UNITED STATES
ENERGY RESEARCH AND
DEVELOPMENT ADMINISTRATION
CONTRACT W-7405-ENG. 36

FLOW-SYSTEM MULTIANGLE LIGHT-SCATTERING STUDIES: A PROGRESS REPORT

by

G. C. Salzman, D. E. Burger,[1] M. Bartholdi,[2] and B. J. Price

Biophysics and Instrumentation Group, Los Alamos Scientific Laboratory
University of California, Los Alamos, New Mexico 87545 USA

INTRODUCTION

In principle, the morphology of a scattering object uniquely determines its light-scattering pattern. This indicates that light scattering might provide a powerful tool both for the analysis of cells and for discrimination of cells from heterogeneous populations. Small-angle light scattering from both particles and biological cells has been shown to be a measure of their size (1-9). A number of authors have demonstrated the utility of light scattering at larger angles for cell discrimination (7,10-16). We present in this paper a progress report on the analysis of particles and tissue culture cells by multiangle light-scattering measurements.

MATERIALS AND METHODS

The instrument is shown in Fig. 1 and has been described in some detail in a previous paper (14). A 3-mW helium-neon laser beam (632.8 nm wavelength) is focused by a 150-mm focal length spherical lens into a saline-filled (n = 1.3345) flow chamber (17) where it intersects a sheath flow encased 20- to 30-μm diameter saline sample stream. Light scattered from a cell in the sample stream is detected by a photodiode array containing 32 individual photodiodes in a concentric ring configuration and a back-angle (176°) detector (18). The current pulse resulting from a light flash on each of the rings is amplified logarithmically and the peak of each pulse sensed and held. These held levels are then multiplexed into an analog-to-digital converter and stored in a computer memory.

The data are processed by a cluster analysis technique described elsewhere (14). The cluster representing the raw scatter patterns from a sample of 10-μm diameter polystyrene latex spheres is shown in Fig. 2. The enclosed area represents a band two standard deviations wide around the average scatter pattern for the group. The ring numbers "Parameter" are related to the true scattering angle shown in Table 1. After the raw data are corrected for detector nonuniformities, detector area, detector distance, laser power, logarithmic amplifier gain, and analog-to-digital converter gain, the patterns should be proportional to the actual scattering intensity. Figure 3 shows the mean of the 10-μm sphere data in Fig. 2 presented as relative intensity vs angle. The three data points between 2° and 3° were removed because of problems with amplifiers. The lines connecting the points are not meant to represent the data. Note that, for this highly refractile sphere (index of refraction relative to water = 1.20), the apparent detailed structure in the raw data (Fig. 2) is preserved in the corrected data (Fig. 3).

---

[1] Associated Western Universities Fellow, New Mexico State University, Las Cruces, New Mexico USA.

[2] Predoctoral Fellow, Clarkson College, Potsdam, New York, and Memorial Sloan-Kettering Cancer Center, New York City, New York USA.

RESULTS AND DISCUSSION

An asynchronous population of Chinese hamster ovary (CHO) tissue culture cells was fixed in 1.5% glutaraldehyde and later resuspended in normal saline. This particular preparation produced a significant number of cells from which the cytoplasm had been removed. This nuclei subpopulation was identified by comparison with a sample containing only CHO nuclei. The raw data clusters are shown in Fig. 4, and the corrected average scatter pattern from each cluster is shown in Fig. 5. Note again the three missing data points between 2° and 3°. For both the whole CHO cells and nuclei, the region between 1° and 2° is well within the diffraction-dominated forward scattering lobe, and the data can be expected to indicate relative cell size. Beyond the forward scattering lobe, contributions due to reflection and refraction dominate the pattern. Therefore, internal structure such as the presence of the nucleus can be expected to contribute significantly to the pattern. One surprising feature of these data is the lack of structure in the pattern. This may be due to the large angular range subtended by the detector elements at larger angles and the low refractive index of the CHO cells relative to water (m = 1.02 to 1.03) (19). Another interesting feature of these data is that the nuclei and whole cell scatter patterns follow each other closely over a broad angular range above 3°.

SUMMARY

Multiangle light-scatter patterns for 10-μm diameter polystyrene latex microspheres and for glutaraldehyde-fixed CHO cells and nuclei are presented as relative scattering intensity vs corrected scattering angle. This short presentation is meant only to be a report of work in progress. The data are preliminary, and work is now under way to compare the microsphere data with exact electromagnetic theory calculations.

ACKNOWLEDGMENTS

This work was supported by the U. S. Energy Research and Development Administration and the U. S. National Cancer Institute under interagency agreement YO1-CB-60311. The authors would like to thank Ms. Zita Svitra and Dr. Marylou Ingram for cell preparation, Mrs. Julie Grilly for photography, and Mrs. Elizabeth Sullivan for typing the manuscript.

REFERENCES

1. P. Latimer and B. Tully, Small-angle scattering by yeast cells. A comparison with the Mie predictions. J. Coll. Interface Sci. 27:475 (1968).
2. P. F. Mullaney, M. A. Van Dilla, J. R. Coulter, and P. N. Dean, Cell sizing: A light scattering photometer for rapid volume determination. Rev. Sci. Instrum. 40:1029 (1969).
3. P. F. Mullaney and P. N. Dean, Cell sizing: A small-angle light-scattering method for sizing particles of low relative refractive index. Appl. Opt. 8:2361 (1969).
4. P. F. Mullaney and P. N. Dean, The small-angle light scattering of biological cells, theoretical considerations. Biophys. J. 10:764 (1970).
5. D. J. Arndt-Jovin and T. M. Jovin, Computer-controlled cell (particle) analyzer and separator. Use of light scattering. FEBS Lett. 44:247 (1974).
6. A. L. Koch, Theory of the angular dependence of light scattered by bacteria and similar-sized biological objects. J. Theor. Biol. 18:153 (1968).
7. A. L. Koch and E. Ehrenfeld, The size and shape of bacteria by light scattering measurements. Biochim. Biophys. Acta 165:262 (1968).
8. M. H. Julius, R. G. Sweet, G. C. Fathman, and L. A. Herzenberg, Fluorescence activated cell sorting and its application, In: Mammalian Cells: Probes and Problems (C. R. Richmond, D. F. Petersen, P. F. Mullaney, and E. C. Anderson, eds.), Technical Information Center, Oak Ridge Tenn., ERDA Symposium Series CONF-731007 (1975), pp. 107-121.
9. M. R. Loken and L. A. Herzenberg, Analysis of cell populations with a fluorescence activated cell sorter. Ann. N. Y. Acad. Sci. 254:263 (1975).
10. L. S. Cram and A. Brunsting, Fluorescence and light scattering measurements on hog cholera-infected PK-15 cells. Exp. Cell Res. 78:209 (1973).
11. R. A. Meyer, S. F. Haase, S. E. Podulso, and G. M. McKhann, Light scatter patterns of isolated oligodendroglia. J. Histochem. Cytochem. 22:594 (1974).
12. A. Brunsting and P. F. Mullaney, Differential light scattering: A possible method of mammalian cell identification. J. Coll. Interface Sci. 39:492 (1972).
13. R. E. Kopp, J. Lisa, J. Mendelsohn, B. Pernick, H. Stone, and R. Wohlers, The use of coherent optical processing techniques for automatic screening of cervical cytologic samples. J. Histochem. Cytochem. 22:598 (1974).
14. G. C. Salzman, J. M. Crowell, C. A. Goad, K. M. Hansen, R. D. Hiebert, P. M. LaBauve, J. C. Martin, M. Ingram, and P. F. Mullaney, A flow-system multiangle light-scattering instrument for cell characterization, Clin. Chem. 21:1297 (1975).
15. G. C. Salzman, J. M. Crowell, J. C. Martin, T. T. Trujillo, A. Romero, P. F. Mullaney, and P. M. LaBauve, Cell classification by laser light scattering: Identification and separation of unstained leukocytes. Acta Cytol. 19:374 (1975).

16. M. R. Loken, R. G. Sweet, and L. A. Herzenberg, Cell discrimination by multiangle light scattering. J. Histochem. Cytochem. 24:284 (1976).

17. J. A. Steinkamp, M. J. Fulwyler, J. R. Coulter, R. D. Hiebert, J. L. Horney, and P. F. Mullaney, A new multiparameter separator for microscopic particles and biological cells. Rev. Sci. Instrum. 44:1301 (1973).

18. G. C. Salzman, B. J. Price, R. D. Hiebert, P. F. Mullaney, and J. W. M. Visser, Laser backscattering measurements on biological cells: Preliminary results, In: Proceedings of the Workshop on Separation of Normal and Neoplastic Cells, sponsored by The Netherlands Society against Cancer, held in Lunteren, The Netherlands (November 9-11, 1976).

19. A. Brunsting and P. F. Mullaney, Differential light scattering from spherical mammalian cells. Biophys. J. 14:439 (1974).

TABLE 1

RELATIONSHIP BETWEEN PARAMETER NUMBER IN FIG. 2 AND ACTUAL SCATTERING ANGLE SUBTENDED

Parameter Number	Mean Angle (°)	Half-Angle Subtended
1	0.00	0.14
2	0.28	0.05
3	0.43	0.06
4	0.58	0.06
5	0.73	0.06
6	0.89	0.06
7	1.05	0.07
8	1.23	0.07
9	1.41	0.08
10	1.62	0.09
11	1.85	0.10
12	3.05	0.17
13	3.46	0.19
14	3.91	0.22
15	4.43	0.25
16	5.01	0.29
17	5.66	0.33
18	6.39	0.37
19	7.21	0.41
20	8.11	0.46
21	9.11	0.51
22	10.22	0.56
23	11.43	0.61
24	12.74	0.67
25	14.17	0.72
26	15.69	0.77
27	17.32	0.82
28	19.03	0.86
29	20.82	0.90

FIGURE LEGENDS

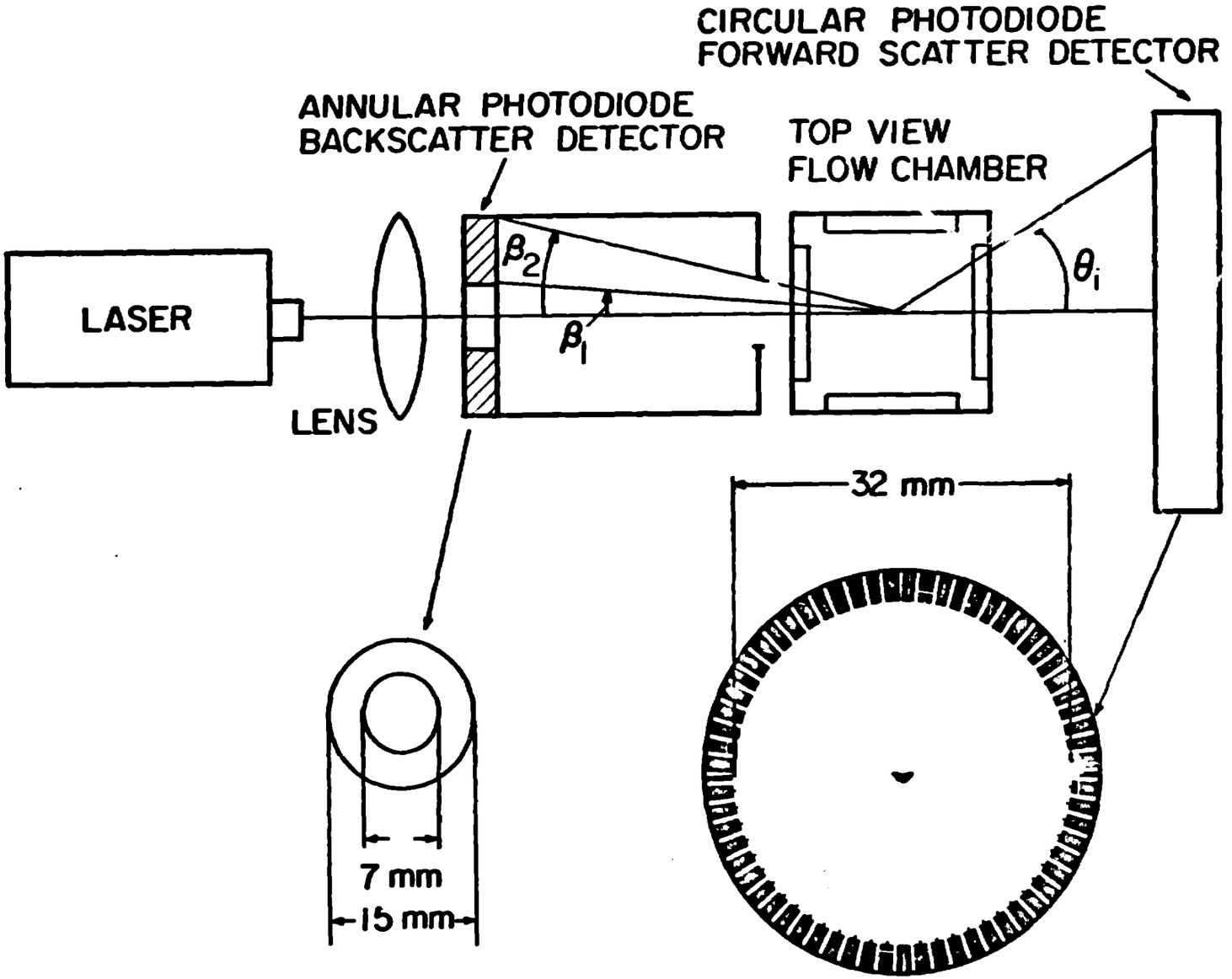
Fig. 1. Schematic drawing of the flow-system multiangle light-scattering instrument. The backscatter detector was not used in these measurements.

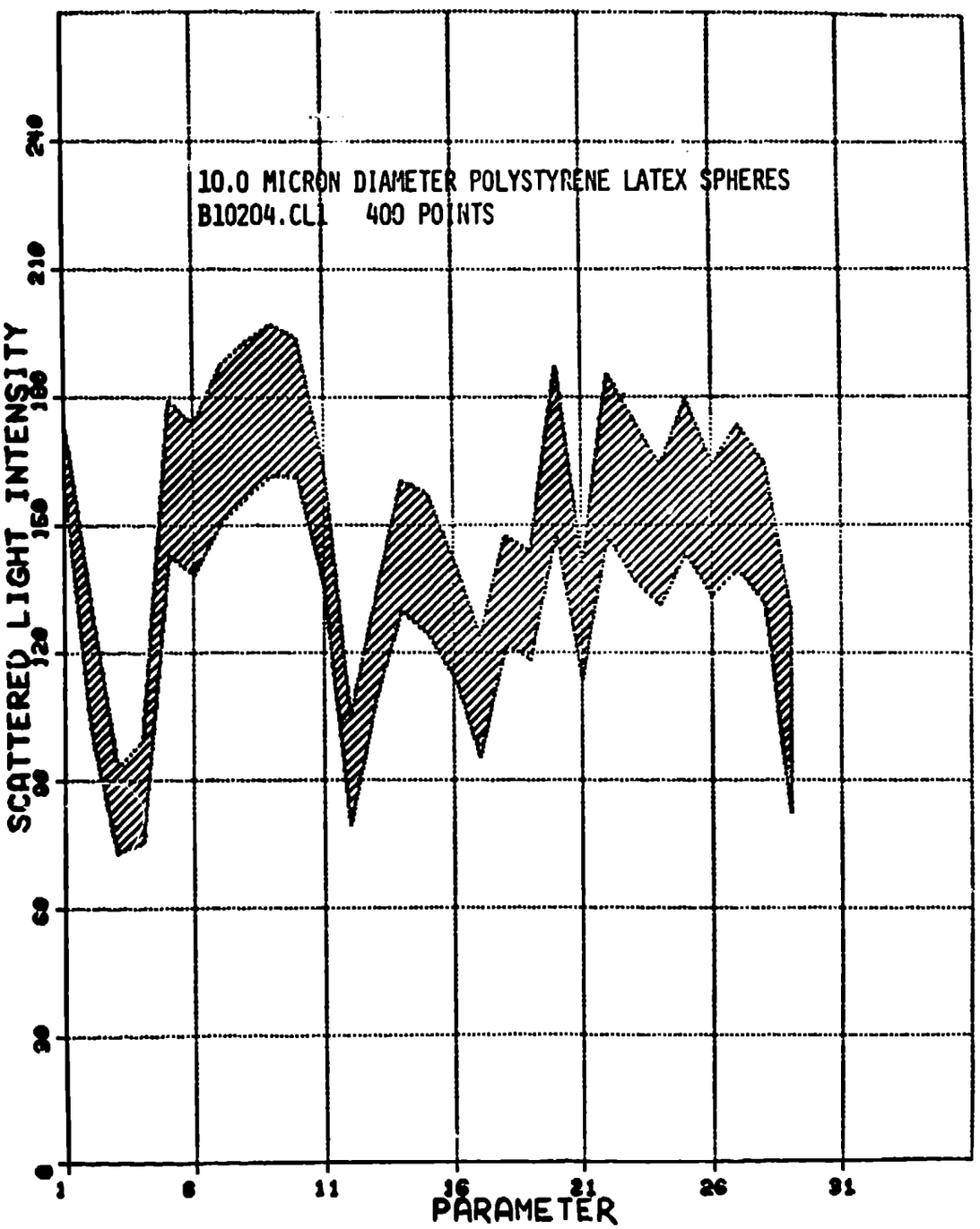
Fig. 2. Cluster representing the raw scatter patterns from a sample of 10-μm diameter latex microspheres. The abscissa gives ring number (see Table 1), and the ordinate gives the logarithm of the uncorrected scattered light intensity. The ordinate numbers are to be ignored.

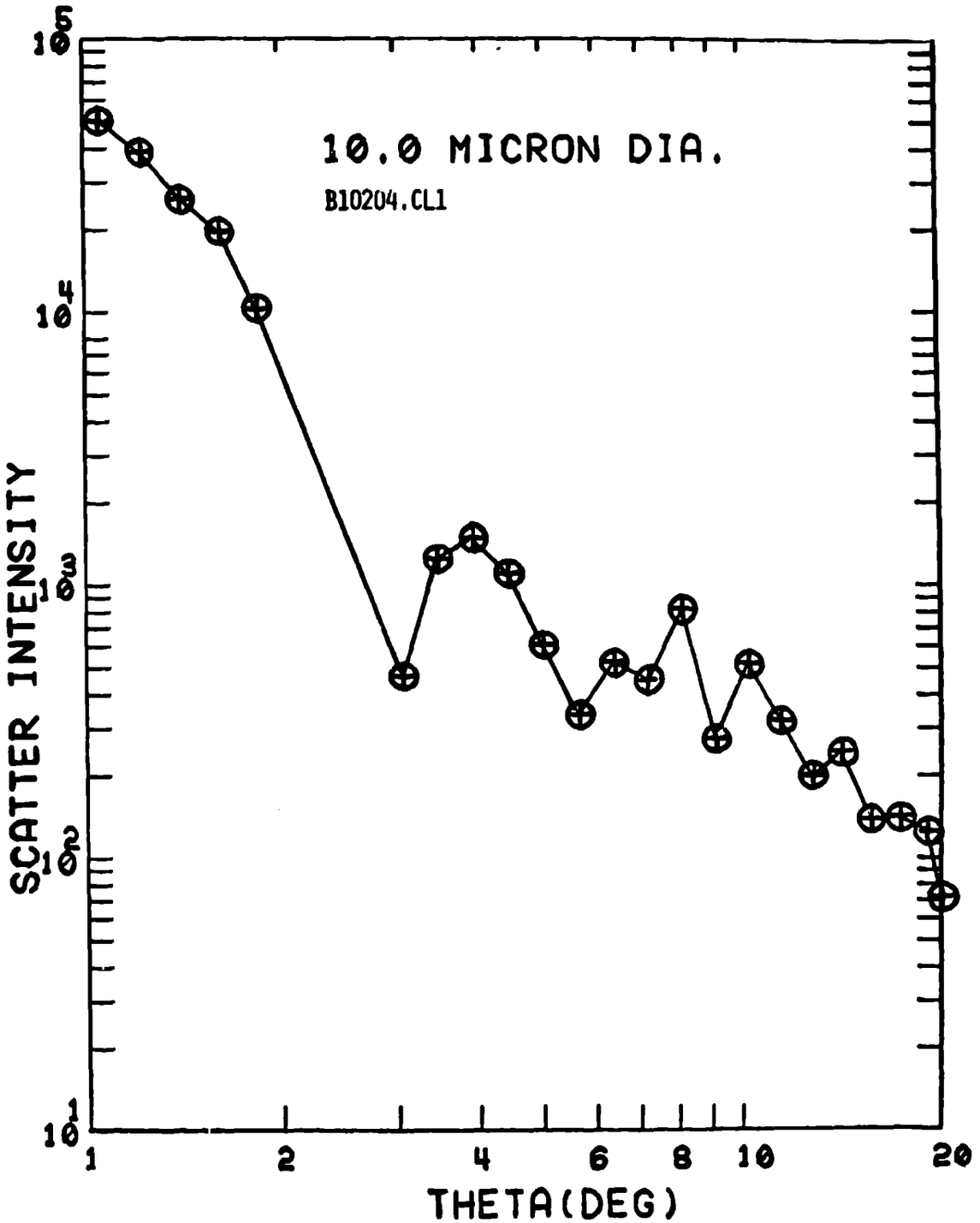
Fig. 3. The average of 400 corrected scatter patterns for the 10-μm sphere data shown in Fig. 2. The abscissa gives true scattering angle (log-scale), and the ordinate gives relative scatter intensity (log-scale). Note that three points are missing between 2° and 3°.

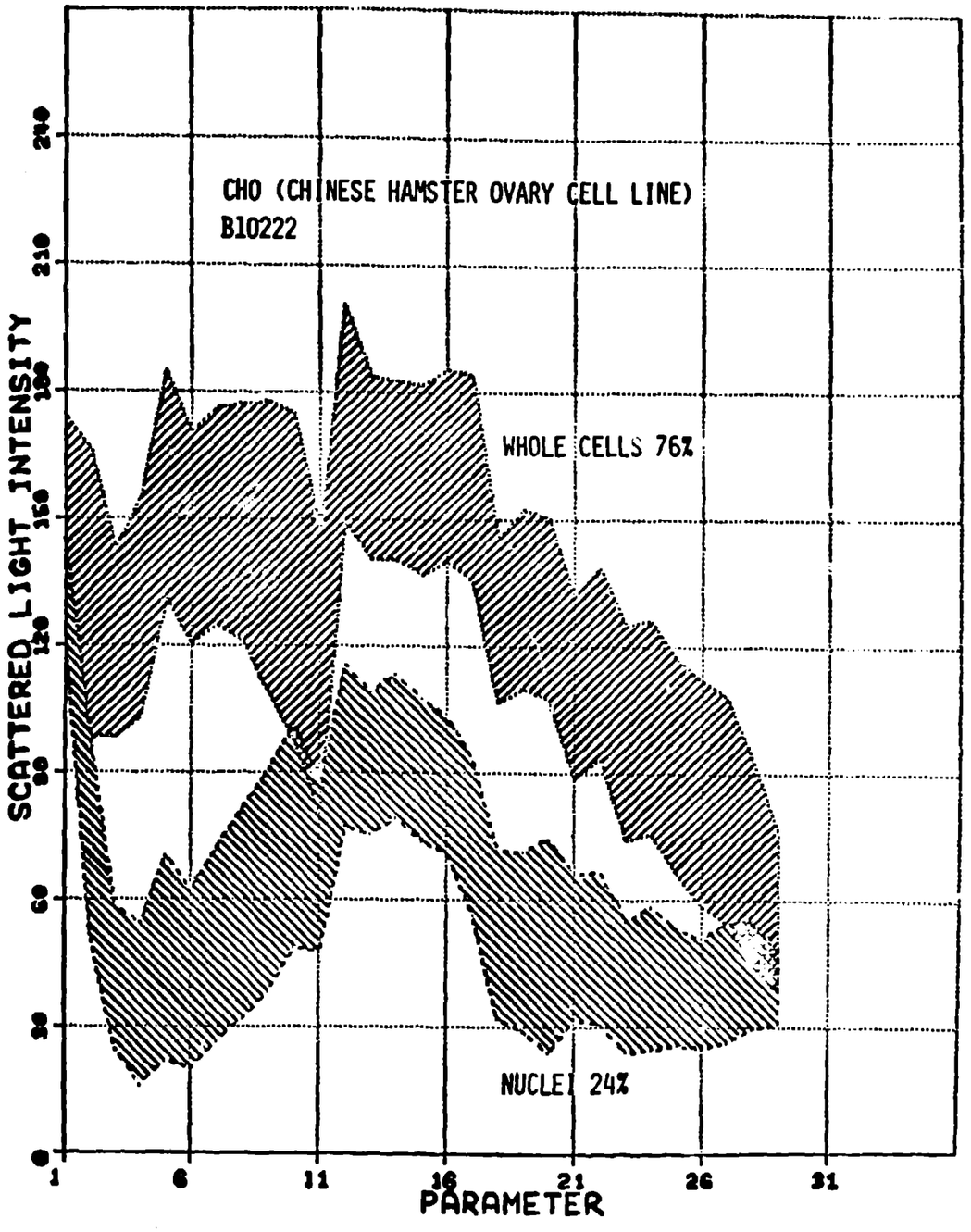
Fig. 4. Two clusters of raw scatter patterns from a sample of Chinese hamster ovary tissue culture cells containing some stripped nuclei. The nuclei were identified by comparison with a sample containing only nuclei.

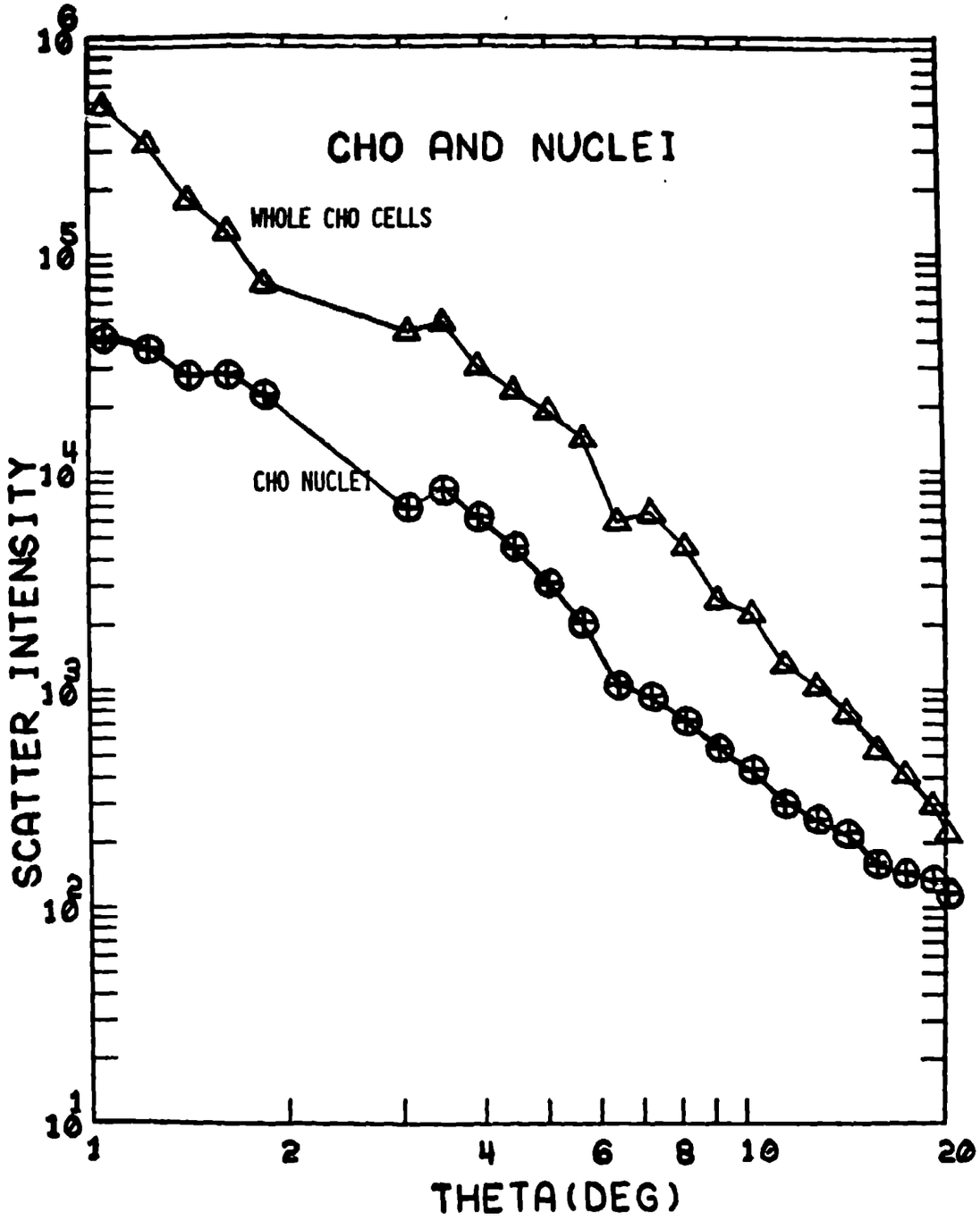
Fig. 5. The average scatter pattern from each of the clusters shown in Fig. 3 corrected to relative scatter intensity. Note the three missing data points between 2° and 3°.


CIRCULAR PHOTODIODE
FORWARD SCATTER DETECTOR
ANNULAR PHOTODIODE
BACKSCATTER DETECTOR
TOP VIEW
FLOW CHAMBER
LASER
LENS
β_2
β_1
θ_i
32 mm
7 mm
15 mm


10.0 MICRON DIAMETER POLYSTYRENE LATEX SPHERES
B10204.CLI 400 POINTS
SCATTERED LIGHT INTENSITY
0
30
60
120
150
180
210
240
1
6
11
16
21
26
31
PARAMETER


10.0 MICRON DIA.
B10204.CL1
SCATTER INTENSITY
THETA(DEG)
10^5
10^4
10^3
10^2
10^1
1
2
4
6
8
10
20


CHO (CHINESE HAMSTER OVARY CELL LINE)
B10222
WHOLE CELLS 76%
NUCLEI 24%
SCATTERED LIGHT INTENSITY
PARAMETER
1
6
11
16
21
26
31


CHO AND NUCLEI
WHOLE CHO CELLS
CHO NUCLEI
SCATTER INTENSITY
THETA(DEG)
1
2
4
6
8
10
20